Remarks

Applicants request re-consideration of the above-referenced patent application.

I. Claim Listing

Claims 1-11 and 16 are pending, and are shown in the previous section. This response does not cancel or amend claims, nor does it add any new claims.

II. Response to the rejection of claims 1-9 and 11 under 35 U.S.C. §103(a)

The Office action rejects claims 1-9 and 11 under 35 U.S.C. §103(a) for being obvious over Heath et al. (U.S. Patent No. 5,235,039), Bromberg (U.S. Patent No. 4,203,670), and Maeda (*Assay of Proteolytic Enzymes by the Fluorescence Polarization Technique*, Anal. Biochem., 92:222-227 (1979)) in view of Welch et al. (*A Herpesvirus Maturational Proteinase, Assembiin: Identification of Its Gene, Putative Active Site Domain, and Cleavage Site*, PROC. NATL. ACAD. SCI., 88:10792-10796 (1991)) or Blakeslee et al. (*Immunofluorescence Using Dichlorotriazyl-aminofluorescein (DTAF). I. Preparation and Fractionation of Labelled IgG*, J. IMMUNOL. METHODS, 13(3-4):305-320 (1976)). Applicants request withdrawal of the rejection. The August 11, 2003 Office action acknowledged that claims 1-11 are patentable over Heath et al. in view of Welch et al. or Blakeslee et al. Applicants submit that adding the teachings of Bromberg and Maeda to the teachings of Heath et al. in view of Welsh et al. or Blakeslee et al. does not render claims 1-9 and 11 obvious because there is no motivation to combine the teachings of those references. Applicants also submit that even if one to combine the teachings of Heath et al., Bromberg, and Maeda in view of Welch et al. or Blakeslee et al., those combined teachings would fail to teach, suggest, or provide motivation for the methods of claims 1-9 and 11.

A. Claim 1

Claim 1 is directed to a method for determining the activity of a protease comprising (a) incubating a mixture of the protease and a substrate capable of being bound to an anchor and having a fluorescent radical attached thereto; (b) binding the substrate to the anchor; (c) measuring the fluorescence polarization of the mixture; and (d) correlating measured fluorescence polarization to protease activity.

Bromberg discusses a system and method for measuring polarization of fluorescence. As acknowledged by the August 11, 2003 Office action, Bromberg does not discuss how to measure protease activity. In fact, Bromberg does not discuss how to measure any enzyme activity. And Bromberg does not discuss any substrates suitable for measuring enzyme activity regardless of whether those substrates can or cannot bind to the anchor of claim 1.

Maeda discusses measuring trypsin digestion of FITC-labeled fibrinogen, papain digestion of FITC-labeled IgG, pepsin digestion of FITC-labeled soybean trypsin inhibitor, and Pronase digestion of FITC-labeled ribonuclease by utilizing a single-cuvette fluorescence polarization assay. Maeda's substrates are whole-length proteins that, unlike the substrates of claim 1, are incapable of binding to an anchor. As explained in the reference, those substrates can be used in the fluorescence polarization assay only because they are cut in the proximity of the FITC-labeled N-terminal end, thus resulting in FITC-labeled substrate fragments with a size much smaller than the initial whole-length protein substrate. Thus, Maeda does not discuss how to vary size and polarization value by binding some of the compounds in the reaction mixture to the anchor of claim 1.

Heath et al. discuss polypeptide HIV-1 protease substrates that have a FITC label on one side of the scissile bond and a biotin label on the other side of the scissile bond. Heath et al. also discuss a fluorescence assay allegedly useful for rapid determination of HIV-1 protease activity in a large number of samples. The Heath et al. assay is a multi-step fluorescence assay that requires the physical separation of the FITC-labeled substrate fragments from the reaction mixture containing the biotin-labeled substrate fragments and the remaining uncut substrate. Contrary to the Office action's assertion, the Heath et al. physical separation of fluorescence-labeled substrate fragments cannot be an additional step in the method of claim 1 because the method of claim 1 was designed as a single-cuvette method that renders unnecessary the separation step described by Heath et al. The method of claim 1 utilizes the differences in size and polarization value of the compounds in the reaction mixture. The fluorescence-labeled substrate fragments of claim 1 do not bind to the anchor; they are lighter, tumble faster than the anchor-bound compounds, and have a lower polarization value than the anchor-bound compounds.

As discussed above, Maeda emphasizes assessing protease activity by utilizing whole-

length proteins as substrates and measuring fluorescence polarization without separating the fluorescent substrate fragments from the rest of the reaction mixture. Heath et al., on the other hand, emphasize assessing protease activity by utilizing polypeptide substrates and measuring the fluorescence of the fluorescent substrate fragments after separating those fragments from the reaction mixture. Thus, Maeda and Heath et al. provide conflicting guidance about the substrates and protease assays used. Further, using the Heath et al. polypeptide substrates in the single-cuvette Maeda assay would likely result in an inoperable assay because the fluorescent substrate fragments and the starting fluorescent substrate would have similar size and polarization values (thus resulting in inaccurate protease activity determinations). Finally, although the Bromberg and Maeda references were available for over 10 years before Heath et al. designed their assay, Heath et al. did not come up with the idea to use biotin-containing substrates in a single-cuvette fluorescence polarization assay, nor did Heath et al. recognize that such an assay could be designed using substrates capable of binding to an anchor. Thus, one skilled in the art would have no motivation to combine the teachings of Bromberg and Maeda with the teaching of Heath et al. And for the same reasons, the combined teachings of Bromberg, Maeda, and Heath et al. also fail to teach, suggest, or provide motivation for the method of claim 1.

Welch et al. provide in Table 2 the recognition and cleavage sites for seven herpes virus proteinase substrates. Those substrates are whole length proteins that, unlike the substrates of claim 1, are incapable of binding to an anchor. Blakeslee et al. state that FITC- and DTAF-labeled antibodies have similar immunofluorescence specificity. One skilled in the art would know, however, that FITC- and DTAF-labeled antibodies may not necessarily perform in a similar manner in a fluorescence polarization assay because it is not uncommon for antibodies that perform well in one type of assay to perform poorly in another type of assay. In addition, FITC- and DTAF-labeled substrates other than antibodies may not necessarily have similar fluorescence properties. For example, Applicants have enclosed an excerpt from the Jackson ImmunoResearch Laboratories Inc.'s website explaining that DTAF-streptavidin and FITC-streptavidin have different fluorescence properties in the presence of biotin. And Blakeslee et al. do not discuss how to measure protease activity, nor do they discuss any substrates suitable for measuring enzyme activity regardless of whether those substrates can or cannot bind to the

anchor of claim 1. Thus, combining the teachings of Bromberg, Maeda, and Heath et al. with the teachings of Welch et al. or Blakeslee et al. does not change the above analysis.

To conclude, it was Applicants (not the cited references) that came up with the idea to assess protease activity in a single-cuvette assay by utilizing substrates of any size that can bind to an anchor (thus generating compounds of different size) and measuring fluorescence polarization without separating the fluorescent substrate fragments from the rest of the reaction mixture. The Office action has engaged in an impermissible hindsight by relying on the teachings from Applicants' specification when assessing the patentability of claim 1. Thus, the rejection to claim 1 should be withdrawn.

B. **Claim 2-10**

Claim 2-10 depend (directly or indirectly) from claim 1, and are therefore patentable over the cited references for at least the same reasons discussed above with respect to claim 1.

C. **Claim 11**

Claim 11 is directed to a method for identifying compounds which inhibit a protease comprising (a) incubating a mixture of the protease, the compound, and a substrate having both a fluorescent radical and a radical capable of binding to an anchor; (b) binding the substrate to the anchor; (c) measuring the fluorescence polarization of the emitted light; and (d) calculating the amount of protease inhibition. Thus, claim 11 should be patentable over the cited references for at least the same reasons discussed above with respect to claim 1.

III. **Response to the objection to claim 10**

The Office action objects to claim 10 for depending from rejected claim 2. Applicants request withdrawal of the objection because, as discussed above, claim 2 is patentable over the cited references.

IV. **Acknowledgement of patentability**

Applicants acknowledge the Office action's determination that claim 16 is allowable.

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Applicants request a two-month extension, and authorize the Commissioner to charge the extension fee to Deposit Account No. **08-0750**. Applicants believe that they do not owe any other fee in connection with this response. If, however, Applicants do owe any such fee(s), the Commissioner is hereby authorized to charge the fee(s) to Deposit Account No. **08-0750**. In addition, if there is ever any other fee deficiency or overpayment under 37 C.F.R. §§1.16 or 1.17 in connection with this patent application, the Commissioner is hereby authorized to charge such deficiency or overpayment to Deposit Account No. **08-0750**.

Applicants submit that the pending claims are in condition for allowance, and request that this application be allowed. Applicants request that the Examiner call the undersigned if any issues arise that can be addressed over the phone to expedite examination of this application.

Respectfully submitted,

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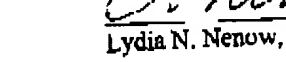

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TechFAQ #6

Why is DTAF-conjugated Streptavidin offered instead of FITC-conjugated Streptavidin?

We have observed that fluorescence from many of our fluorophore-conjugated streptavidins in solution was enhanced following the addition of a saturating amount of free biotin. Of particular significance was the difference we observed between DTAF- and FITC-conjugated streptavidins. Fluorescence from FITC-streptavidin was extremely low when no biotin (curve F-S) was bound to the conjugate. However, after addition of a saturating concentration of free biotin (curve F-S+B) there was a 16-fold increase in fluorescence (Figure 1). A similar response from DTAF-streptavidin was less dramatic (a 1.9-fold increase) (curves D-S and D-S+B), however its fluorescence was much higher than that from FITC-streptavidin before the addition of biotin.

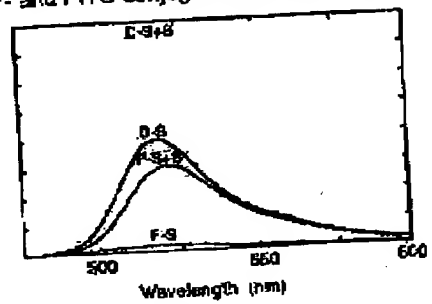

Figure 1

Of more importance, however, was the observation that DTAF-streptavidin was more than twice as fluorescent as FITC-streptavidin before (curves F-S and D-S) and after (curves F-S+B and D-S+B) the addition of free biotin (Figure 1). Although this difference may be somewhat less when the conjugates are bound to only one or two biotins on an antibody or other molecule, these observations suggest that DTAF-streptavidin should be a better fluorescein labeling reagent than FITC-streptavidin. We have therefore added DTAF (code number 016-010-084) to our list of streptavidin conjugates.

Unlike streptavidin fluorescein conjugates, DTAF-conjugated *antibodies* are the same or less fluorescent than FITC-conjugated antibodies.

2/22/2005